A Hypothesis on Biological Protection from Space Radiation Through the Use of New Therapeutic Gases

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Abstract. Radiation exposure to astronauts could be a significant obstacle for long duration manned space exploration because of current uncertainties regarding the extent of biological effects. Furthermore, concepts for protective shielding also pose a technically challenging issue due to the nature of cosmic radiation and current mass and power constraints with modern exploration technology. The concern regarding exposure to cosmic radiation is the biological damage it induces. As damage is associated with increased oxidative stress, it is important and would be enabling to mitigate and/or prevent oxidative stress prior to the development of clinical symptoms and disease. This paper hypothesizes a "systems biology" approach in which a combination of chemical and biological mitigation techniques are used conjunctively. It proposes using new, therapeutic, medical gases as both chemical radioprotectors for radical scavenging and biological signaling molecules for management of the body's response to exposure. From reviewing radiochemistry of water, biological effects of CO, H_2, NO, and H_2S gas, and mechanisms of radiation biology, it is concluded that this approach may have great therapeutic potential for radiation exposure. Furthermore, it also appears to have similar potential for curtailing the pathogenesis of other diseases in which oxidative stress has been implicated including cardiovascular disease, cancer, chronic inflammatory disease, hypertension, ischemia/reperfusion injury, acute respiratory distress syndrome, Parkinson's and Alzheimer's disease, cataracts, and aging.

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Overview - Evolution of Hypothesis

1. Define the Problem
   - What is space radiation?
   - Why is it a challenge?
   - What is radiation damage?

2. Introduce the Hypothesis by Describing its Evolution
   - Is radiolysis of water related to radiation damage?
   - Could mitigation techniques in radiation chemistry of water be applied biologically to mitigate radiation damage?

3. Provide Overview of Some Medical Gas Research
   - Is the idea reasonable & feasible?

What is Space Radiation & Why Is it a Challenge?

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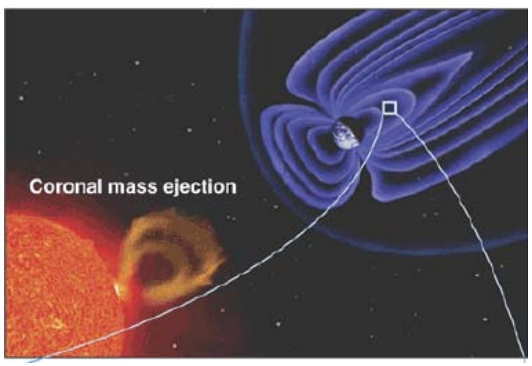
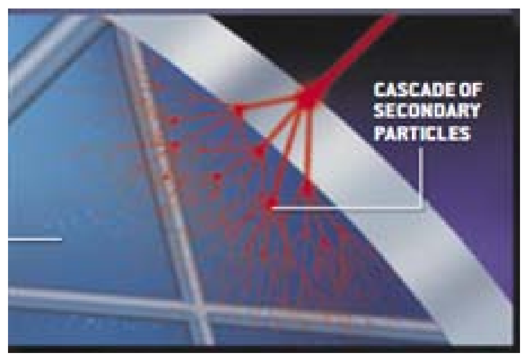
Highly energetic, light to heavy nuclei (high nuclear charge) (HZE) from stars (solar wind or galactic cosmic rays)

NATURE

- highly ionizing & penetrating
- capable of generating 2nd radiation by nuclear fragmentation
- causes biological damage that is more difficult to repair





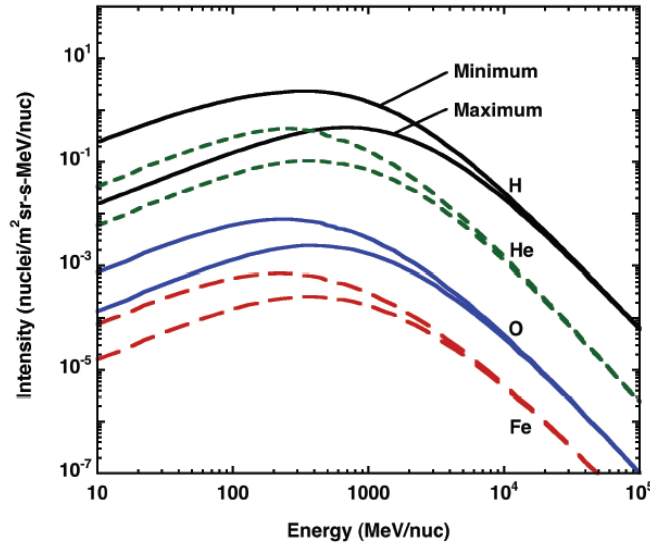


FIGURE 1.1.1 Representative galactic cosmic ray ion spectra depicting the intensity variations between solar maximum and solar minimum conditions. The upper curve for each species is for solar minimum, when cosmic rays can penetrate into the inner heliosphere more easily. SOURCE: Courtesy of R.A. Mewaldt, California Institute of Technology.

CHALLENGES

- difficult to shield (mass attenuation or force deflection (magnetic or electrostatic))
- unpredictability of true exposure & uncertainty in biological risk (currently)
- *w/ faster speeds from advanced propulsion; could relative velocity change the effective energy & thus dose?*

What is Radiation Damage?

How Exposure Causes Disease

Disease is Macroscopic Effects of Molecular Modifications Initiated by Radiation Induced Chemical Reactions

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RADIATION INDUCED DAMAGE & PROPOGATION → **EFFECTS PROPOGATE INTO SYMPTOMS**

CHEMICAL CHANGES
- Ionizes & breaks bonds of molecules
- Makes organic molecules chemically reactive

$\leq 10^{-15}$ sec

MOLECULAR TRANSFORMATION
- reactions cause structural modifications of molecule

MACRO MOLECULAR CHANGES
- structural changes alter molecular properties
- changes biochemical functionality

$\leq 10^{-3}$ sec

CELLULAR CHANGES
- increased membrane permeability
- chromosome aberrations

minutes

TISSUE, ORGAN & SYSTEM CHANGES
- tissue necrosis
- bone marrow damage
- decrease red & white blood cell counts
- etc.

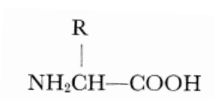
days - weeks

MEDICAL SYMPTOMS
- cancer
- ulcers potentiating infection
- nausea
- anorexia
- infection
- etc.

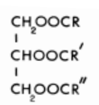
days - years

ENZYMES

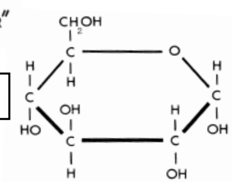
PROTEINS



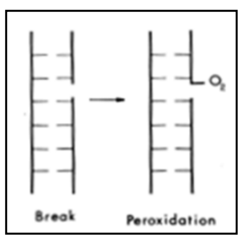
LIPIDS

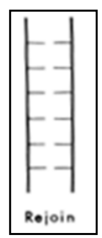


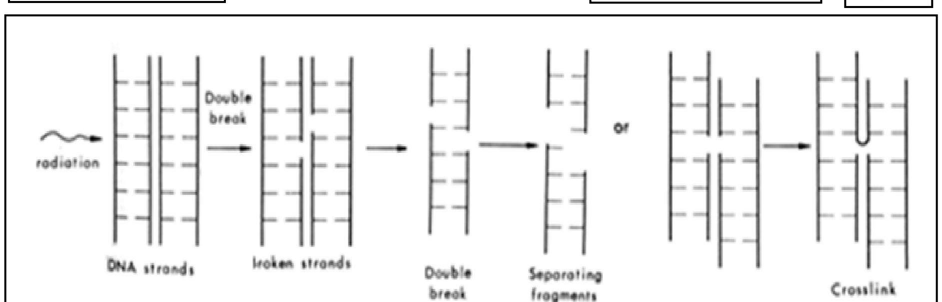
CARBOHYDRATES

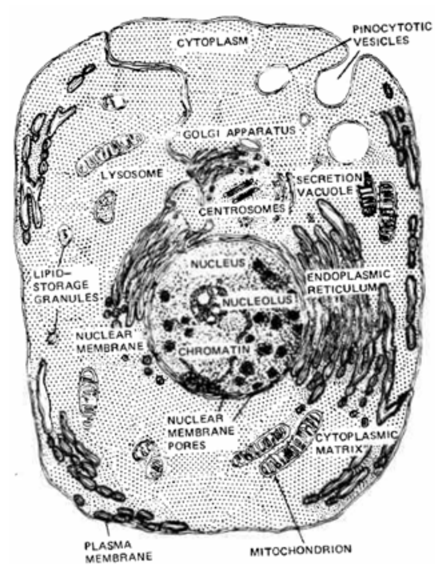


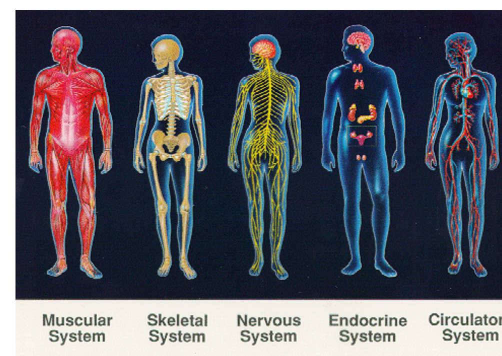
NUCLEIC ACIDS











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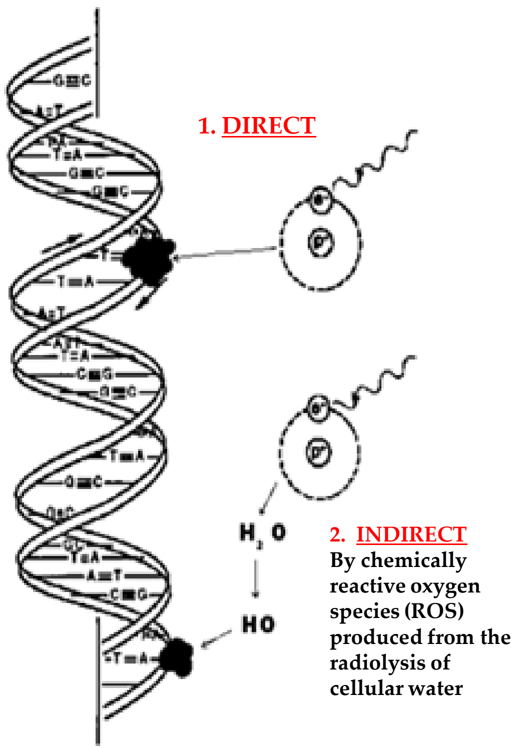
Types of Molecular Damage



80% (water), 5% (DNA), 10-20% (RNA), remainder (protein)

Elkind, M.M., "*Introduction to The Biology of The Mammalian Cell*"", in Physical Mechanisms in Radiation Biology, USAEC conference 721001 Oct. 11-14 1972.

- **Indirect damage is mostly caused by ROS radiolysis byproducts**
  - *"ions will probably have little effect as the DNA contains numerous ionizable positions at the phosphate group."*
  - *"Excited hydrolysis products may transfer the excitation energy to the DNA, leading to a localized break in the sugar-phosphate chain."*
  - *"Free radicals like OH and oxidizing products like H_2O_2 are highly reactive and can add to unsaturated bonds which upsets the sensitive hydrogen-π-bonding and may break the bonding between two helices."*
- **Indirect damage is due to 'poisoning' of cell w/ decomposition products**
  - *"The matrix effect considers the particle-water interaction in which ions, radicals and excited atoms are produced. This is the dominating effect at large radiation does and dose rates…Free radicals and oxidizing products interact directly with cell DNA, causing the DNA-strands to break. One can state that at such high does the cell is simply poisoned by decomposition products and the whole organ may be destroyed."*

    Chopping, G., Liljenzin, J., Rydberg, J., "*Radiochemistry and Nuclear Chemistry*", Butterworth-Heinemann, 3rd. ed., 2002.
  - *"Cross linking process is thought to be primarily a direct effect of the radiation, while double-chain breaks are largely indirect."*
- **Direct damage is non-negligible in a biological systems where there are large molecules**
  - *"… an excess of water in dilute solutions of DNA, however, the indirect effect predominates and double chain breaks are produced…."*
  - *"when aqueous organic solution is irradiated, the usual "indirect" reaction on the organic molecule is the removal of either a H atom or an entire radical group (such as the $–CH_3$ "*
  - *"Normally, in dilute solutions of small molecules, the radiation dose that will cause a considerable proportion of the solute to react with free radicals will only suffice to ionize directly a negligible proportion of the solute molecules. Thus, the direct action of the radiation on the solute molecules is small."*
  - *"However, the fraction of the total reactions which are related to the direct effect can be increased in several ways. If material is irradiated dry, the water molecules have been removed so that there will be only direct interactions with the molecules of the material. If a solution is frozen, the mobility of the radicals which are produced in the water molecules is decreased. This will decrease the possibility of indirect action and result in a greater proportion of the interactions being of the direct type."*
  - *"The dose required to produce a chemical change in a given proportion of the molecules of a substance, by direct action, is inversely proportional to the molecular weight of the substance assuming that the ionic yield is constant. (the larger molecules are more likely to be in the path of the radiation) ….*
  - *The direct effect is not very important in consideration of simple chemical systems, but is of importance in macromolecular and biological systems because of the presence of many large molecules."*
- **Damage among types of biological molecules appears to largely involve loss of H atoms**
  - *"when aqueous organic solution is irradiated, the usual "indirect" reaction on the organic molecule is the removal of either a H atom or an entire radical group (such as the $–CH_3$ "methyl" group) from the molecule"*
  - *"saturated hydrocarbons probably undergo a hydrogen extraction & are converted into alcohols in a two step process"*
  - *"acetic acid most frequently loses a hydrogen atom…"*
  - *"energy which is absorbed any place in the molecule can be transmitted down the molecular chain to the weakest bond….They hydrogen bonds are among the weakest in the molecule and thus, are the first to be broken by radiation."*

Casarett, A.P., "*Radiation Biology*", Prentice-hall, Inc., New Jersey, 1968.

Could radiochemical methods in inhibiting the radiolysis of water be applied biologically to mitigate damage?

Comparing Trends in Radiolysis of Water & Biological Factors of Radiosensitivity

Radiolysis Process

Dissociation of Water by Ionizing Radiation

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Energy Deposition

Mechanisms
- ionization
- excitation

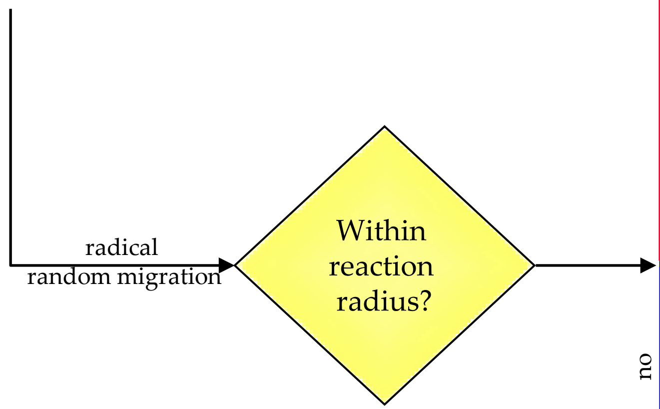
Yields
- ions (H_2O^+, sub-excitation electrons (e^-)
- excited molecules (H_2O^*)

$\leq 10^{-15}$ sec

Free Radical Formation

Mechanisms

1. ionization — $H_2O^+ + H_2O \rightarrow H_3O^+ + OH$
   charge neutralization — $H_3O^+ + e^- \rightarrow 2H + OH$
2. decomposition — $H_2O^* \rightarrow H + OH$ (10^{-14} sec)
   ionization — $H_2O^* \rightarrow H_2O^+ + e^-$
3. thermalization — $e^- \rightarrow e^-_{aq}$ (10^{-12} sec)

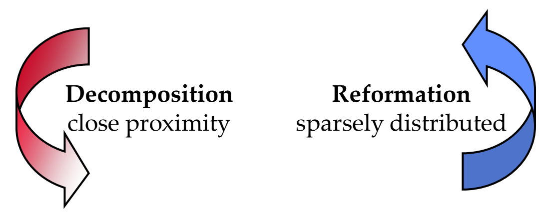


10^{-15} - 10^{-12} sec

Chemical Reactions

radical-radical rx.

like—like ⇔ water decomposition
- ➢ transformation into molecular decomposition products (H_2O_2, O_2, H_2, etc.)
- ➢ alters electrochemical nature of water
- ➢ potential for (1) corrosion (2) pressure rise



radical—molecular decomposition product RXs
- ➢ water reformation
- ➢ reduces potential (1) corrosion (2) pressure rise.

Radicals diffuse into bulk of water

10^{-6} sec

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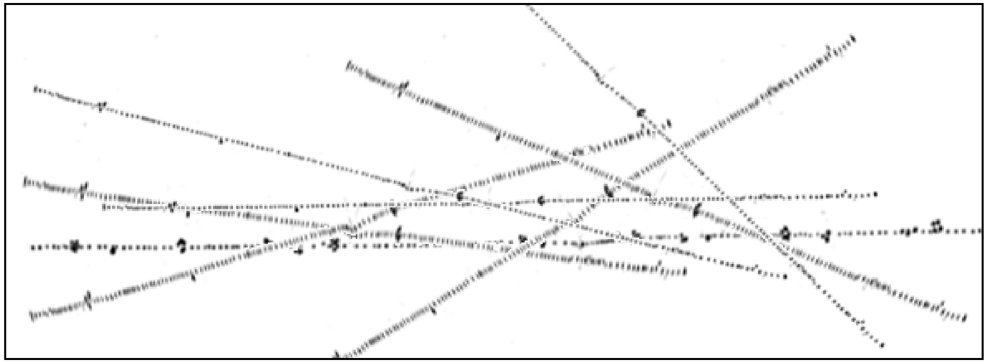
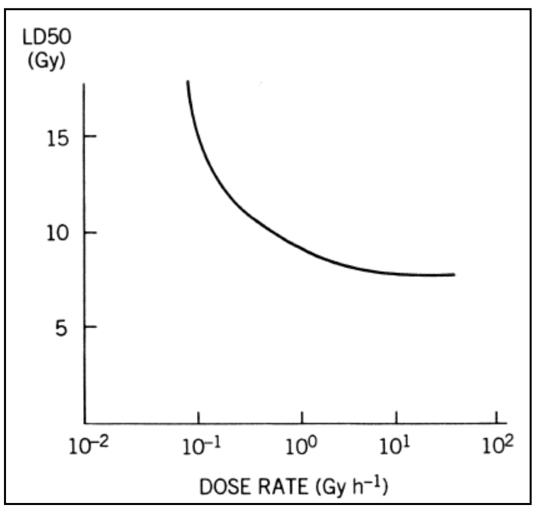
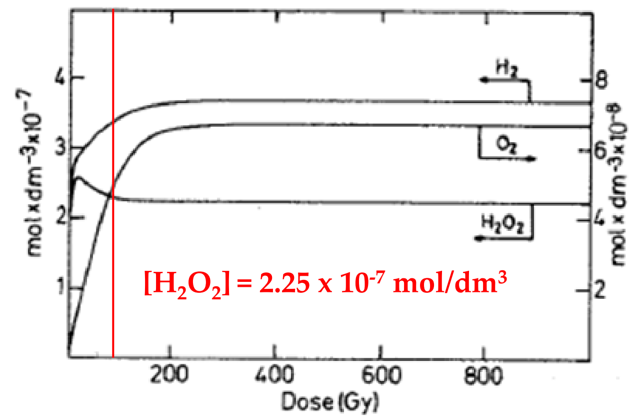
↑ Dose Rate Leads = ↑Water Decomposition ⇔ ↑Higher Lethality



- (+) frequency of particle tracks
- (+) over lapping of particle tracks
- (+) probability of radical-radical rx

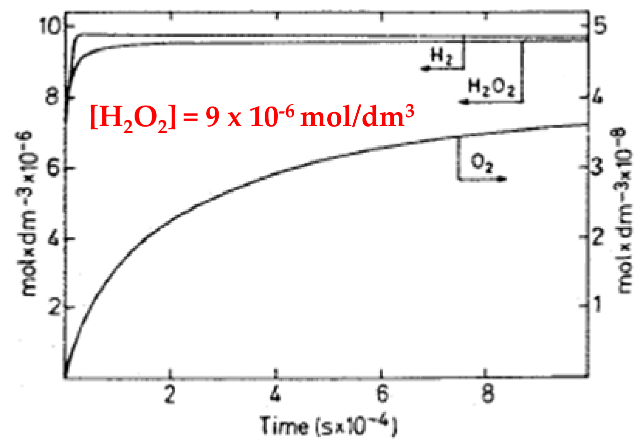


Turner, James E., *Atoms, Radiation, and Radiation Protection*. John Wiley & Sons, Inc., 2nd ed., 1995. pp. 421-422.



100 Gy
in 100 sec

Fig. 1. Radiolytic products in air-free pure water. Dose rate: 1 Gy per second. Doses up to 1000 Gy.



100 Gy
in 1μs

Fig. 2. Formation of radiolytic products in air-free pure wa following a 100 Gy electron pulse delivered in 1×10^{-6} secon

Bjergbakke, E., Draganic, Z. D., Sehested, K., and Draganic, I.G., "Radiolytic Products in Waters Part I: Computer Simulation of Some Radiolytic Processes in the Laboratory," in *Radiochimica Acta*, vol. 48, Munchen, 1989, pp. 66.

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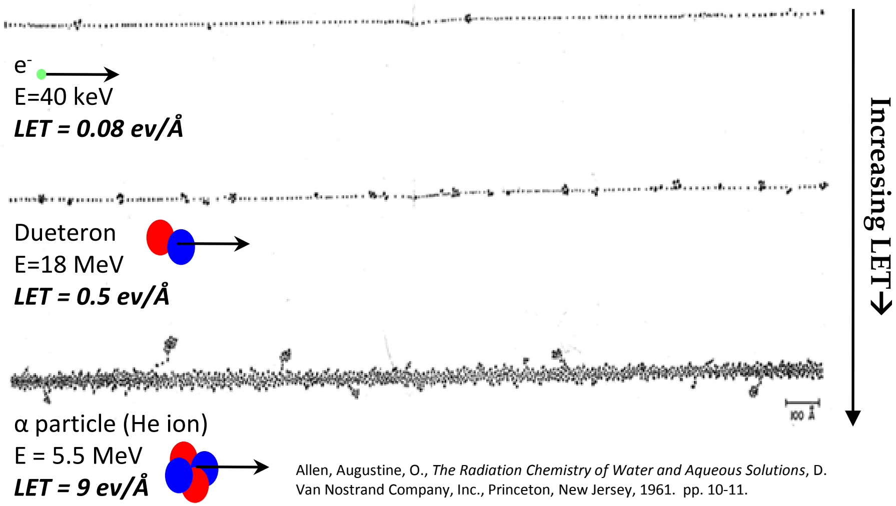
Radiation w/ ↑Linear Energy Transfer (LET) = ↑Water Decomposition ⇔ ↑Damage Efficiency



Allen, Augustine, O., *The Radiation Chemistry of Water and Aqueous Solutions*, D. Van Nostrand Company, Inc., Princeton, New Jersey, 1961. pp. 10-11.

- (+) higher energy deposition locally
- (+) radical production locally
- (+) radical packing density within the tracks & spurs
- (+) probability of radical-radical rx

1. **Radiation with Higher LET has more RBE (relative biological effectiveness)** *[less dose of higher LET rad. is needed to cause given damage]*
2. **higher LET produces type of damage that is more difficult to repair**

- "Low LET radiation sometimes form clusters of ions along the particle track, i.e. produces high LET *spots…High LET spots therefore increase the possibility of damage to both strands of the helix, causing a double strand break*…In addition to double strand breaks being more difficult to repair than single strand breaks, *double strand breaks caused by high LET radiation are more difficult to repair*….It is believed that most single strand breaks are correctly repaired…. *Nevertheless, the repair is more difficult and the chances of "repair errors" (mutations) are much larger than for the single strand break*….*The chromosome aberrations only occur after double strand breaks*."

Chopping, G., Liljenzin, J., Rydberg, J., "Radiochemistry and Nuclear Chemistry", Butterworth-Heinemann, 3rd. ed., 2002.

Impurities Affect the Net Outcome of Chemical Reactions by Competitively Scavenging Radicals

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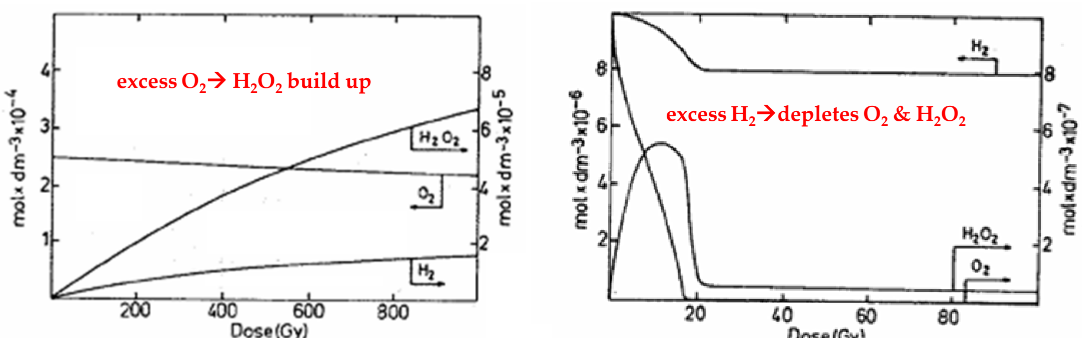
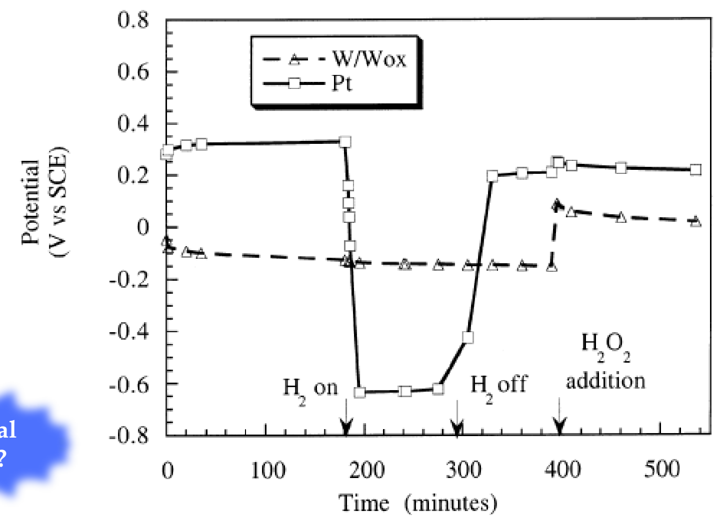
- **Ionic & dissolved gases alter the chemistry by scavenging radicals.**
  - *"…in general, the presence of suspended or colloidal impurities does not result in increased decomposition rates or equilibrium concentrations of decomposition products."*
    Monson, H.O., "Water Decomposition," *The Reactor Handbook*, vol. 2, U.S. Atomic Energy Commission, U.S. Government Printing Office, Washington D.C., 1955, pp. 184.
  - *"…in general, the presence of ionic impurities results in increased decomposition rates and equilibrium concentrations of decomposition products, some impurities producing slight increase and other producing very large increases."*
    Monson, H.O., "Water Decomposition," *The Reactor Handbook*, vol. 2, U.S. Atomic Energy Commission, U.S. Government Printing Office, Washington D.C., 1955, pp. 182.
  - *"At low temperatures, some ionic impurities such as KBr, KI, and CuSO4 may produce partial pressures of 1,500 psi under radiation conditions that produce only a partial pressure of less than 10 psi for relatively pure water. At high temperature, i.e., above 400°F, exploratory work has shown that certain impurities strongly catalyze the backward reaction. Such impurities are copper, rhodium, palladium, platinum, silver, and iodine; and tin, iron, and titanium to a lesser extent."*
    Calkins, Vincent P., "Radiation Damage To Liquids and Organic Materials," *Nuclear Engineering Handbook*, edited by Etherington, Harold, McGraw-Hill Book Company, Inc., New York, 1958, pp.10-132.
- **Certain drugs provide radioprotection by acting as chemical modifiers in scavenging radicals.**
  - *"A number of radiosensitizing chemicals and drugs are known. Some sensitize hypoxic cells, but have little or no effect on normally aerated cells. Other agents known as radioprotectors reduce biological effectiveness….which scavenge free radicals."*
    Turner, James E., *Atoms, Radiation, and Radiation Protection*. John Wiley & Sons, Inc., 2nd ed., 1995. pp. 421-422.

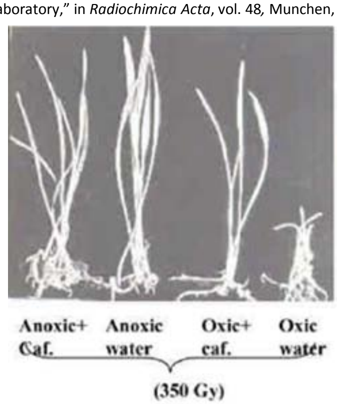


Bjergbakke, E., Draganic, Z. D., Sehested, K., and Draganic, I.G., "Radiolytic Products in Waters Part I: Computer Simulation of Some Radiolytic Processes in the Laboratory," in *Radiochimica Acta*, vol. 48, Munchen, 1989, pp. 66.



Lillard, R.S., Pile, D.L., Butt, D.P., "The Corrosion of Materials in Water Irradiated by 800 MeV Protons," *Journal of Nuclear Materials*, **278**, 200, pp. 277-289.

Kesavan, P.C., "Oxygen effect in radiation biology: Caffeine and serendipity" Current Science, Vo. 89, No.2 25 July 2005. pp. 318-328.



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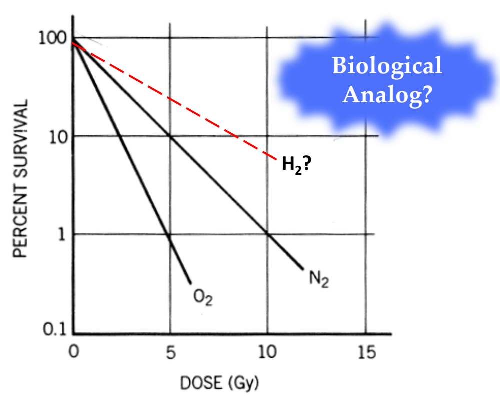
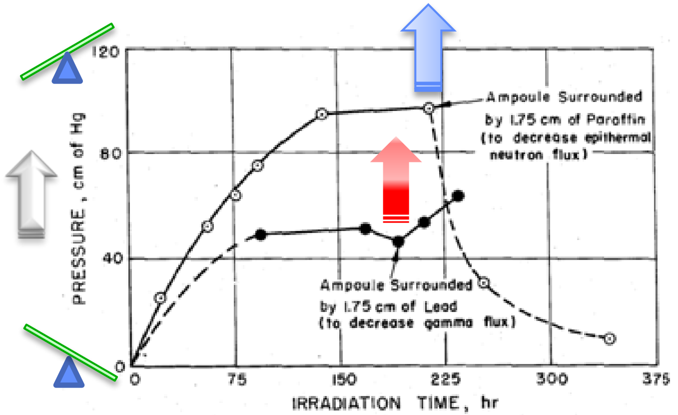


Similar Systematic Behavior: 'Competing Processes w/ Critical Point'?



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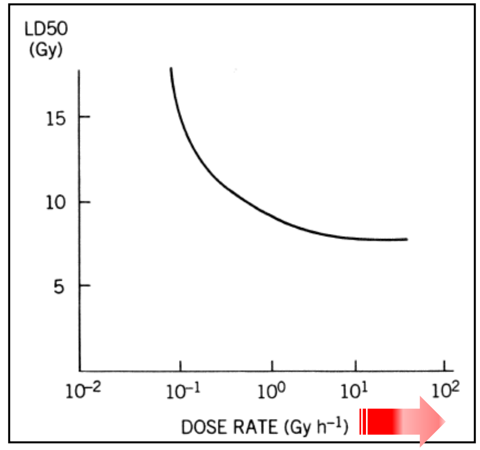

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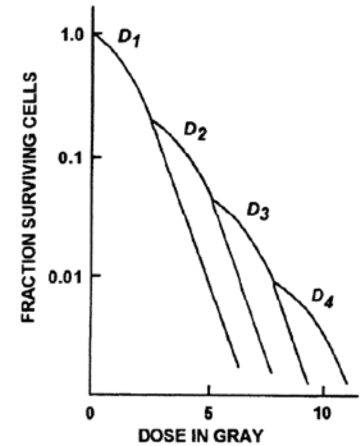
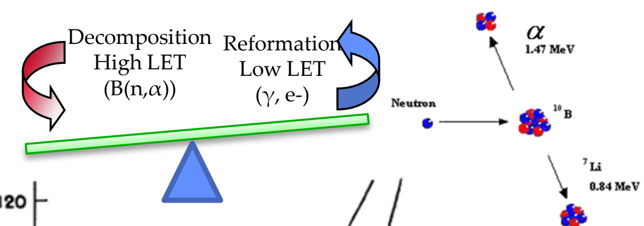
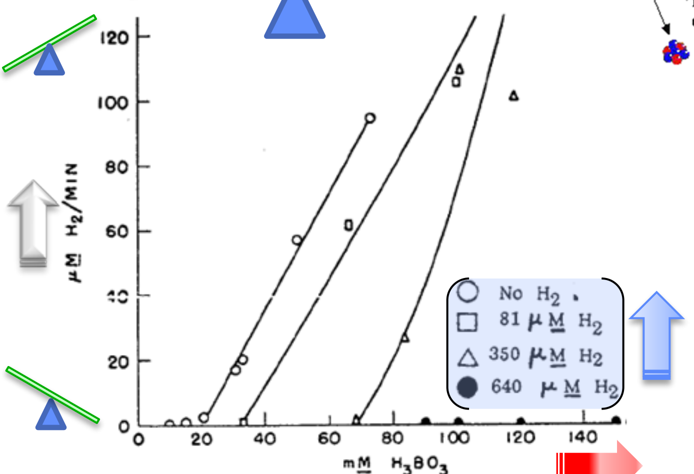
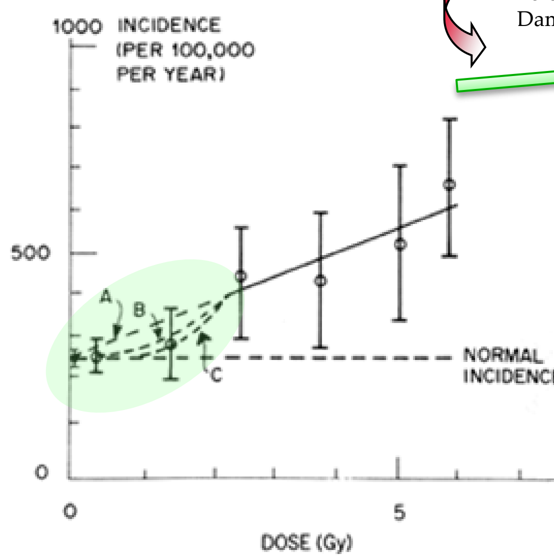
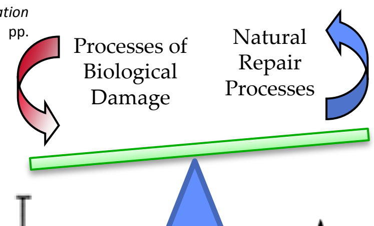






Hart, E.J., McDonell, W.R., and Gordon, S., "The Decomposition of Light and Heavy Water Boric Acid Solutions by Nuclear Reactor Radiations," in proceedings of *International Conference on the Peaceful Uses of Atomic Energy*, Geneva 1955, P/839, Vol. 7, United Nations, New York, 1956, pp. 597.





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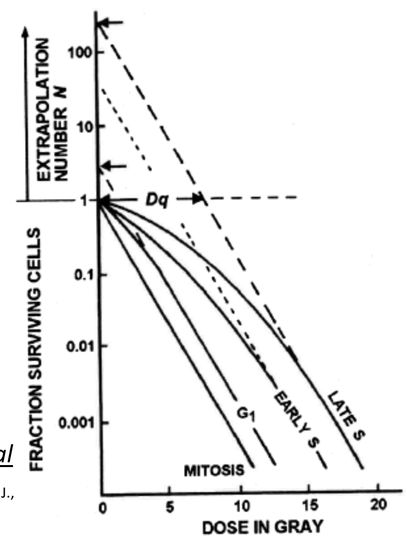

__The scavenging capacity may differ from individual to individual depending on his/her physical condition."__ Chopping, G., Liljenzin, J., Rydberg, J., "*Radiochemistry and Nuclear Chemistry*", Butterworth-Heinemann, 3rd. ed., 2002.

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H_2, CO, & H_2S Medical Gas Countermeasure to Support & Supplement Our Natural Repair System to Increase Tolerance Before Damage Causes Disease

Exposure — DAMAGE PATHWAY — *Disease*

Mitigation Of Chemical Changes by Direct & Indirect Ionizations

- SHIELDING – mass attenuation & force deflection (magnetic, electrostatic)
- prevent uptake radioactive isotope (i.e. I tablets)
- natural cell insensitivity (non-critical cell component, mitotic rate, cycle stage)
- DNA repair mechanism
- radical scavenging by natural antioxidant enzymes
- **temporarily & reversibly inhibit cell cycle to increase time for DNA repair**
- **repair organic radicals**
- **increase antioxidant radical scavenging capacity**
- **decrease radical generation**

Mitigation of Mutations

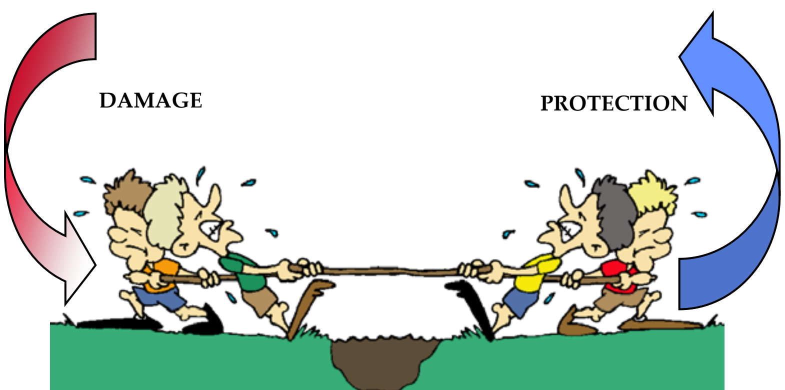
- triggering of apoptosis of mutated cells
- natural DNA repair mechanisms
- apoptosis (cell death)
- Non harmful modification

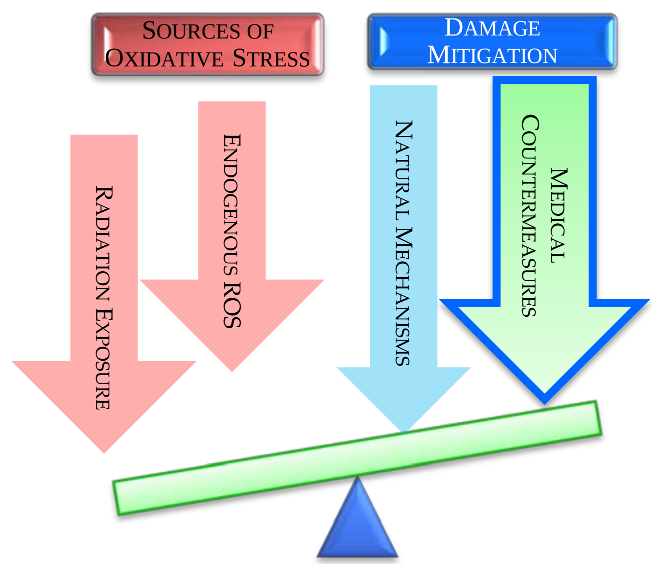
Mitigation of Biological Response

- medical treatments
- **regulation of Immune system response (anti-inflammatory)**

Mitigation of Disease

- radiotherapy
- surgery





Is this feasible?

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Preliminary Compelling Evidence

- Natural repair mechanisms exist & can be supported by various biochemical mechanisms: (1) scavenging, (2) modifying radiosensitivity, & (3) biological promoters/'signaling'/triggering processes
  - *"The cell contains natural radical scavengers. As long as they are in excess of the radiolysis products, the DNA may be protected. When the products exceed the amount of scavengers, radiation damage and cancer induction may occur. In principle, there could thus be a threshold dose for radiation damage, at which the free radicals formed exceed the capacity of scavenging."*

    Chopping, G., Liljenzin, J., Rydberg, J., "*Radiochemistry and Nuclear Chemistry*", Butterworth-Heinemann, 3rd. ed., 2002.
  - *"Also, chemical protectors can be introduced into the system which will compete successfully for the OH and H radicals formed. This will reduce the indirect effect"*

    Casarett, A.P., "*Radiation Biology*", Prentice-hall, Inc., New Jersey, 1968.
  - *"A number of radiosensitizing chemicals and drugs are known. Some sensitize hypoxic cells, but have little or no effect on normally aerated cells. Other agents known as radioprotectors reduce biological effectiveness….which scavenge free radicals. Still other chemicals modifiers have little effect on cell killing but substantially enhance some multistep processes, such as oncogenic cell transformation. For carcinogensis or transformation, such biological promoters can dwarf the effects of physical factors (on dose-response relationships) such as LET and dose rate,"*

    Turner, James E., *Atoms, Radiation, and Radiation Protection*. John Wiley & Sons, Inc., 2nd ed., 1995. pp. 421-422.
- Multiple DNA repair mechanisms exist
  - *"The cell is protected by different DNA repair mechanism which try to restore the damage. We don't know the details, except when the repair goes wrong (e.g. a replacement of a lost nucleotide by a 'wrong" base pair, etc.)…"*

    Chopping, G., Liljenzin, J., Rydberg, J., "*Radiochemistry and Nuclear Chemistry*", Butterworth-Heinemann, 3rd. ed., 2002.
  - *"DNA damage response involves (a) removal of DNA damage & restoration of DNA duplex continuity (b) activation of DNA damage check points that arrests cell cycle (c) transcriptional responses that change profile in potentially beneficial way to cell & (d) apoptosis or cell death of un-repairable DNA.*

    Sanca, A. et. al, "*Molecular Mechanisms of Mammalian DNA Repair and The DNA Damage Checkpoints,*" Annu. Rev. Biochem. 2004. 73:39-85.
- Natural repair appears to be more effective in-vivo
  - *"The repair system is believed to be more effective in a living organism, where the cells are in continuous exchange with the surrounding cells and body fluids, than in the tissue samples often studied in the laboratory…"*

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Evidence Among Various Fields

- **Radio-sensitivity & protection ⇔ Effect of Dissolved Gases on Radical Scavenging**
  - **Water Chemistry** - ionic & dissolved gas impurities scavenge free radicals promoting various outcomes depending upon type
  - **Radiobiology** - some radioprotectors function by scavenging radicals
  - **Health** - role of antioxidants in disease prevention
  - **Water Chemistry** - O_2 promotes water decomposition & formation of H_2O_2
  - **Radiobiology** - O_2 is a radiosensiziter & causes peroxidation damage of DNA. Also, tissue hypoxia has been attributed w/ radioprotective effects
  - **Medical Gases** - CO appears to mitigates oxidative stress
  - **Water Chemistry** - H_2 promotes water reformation, inhibits formation of H_2O_2, & reduces open circuit potential of water
  - **Radiobiology** - repair of organic radicals by H atom donation demonstrated in polymers
  - **Medical Gases** - H_2 appears to mitigate oxidative stress

- **Similarities in systematic behavior of 'competing processes' in which critical point of process domination can be altered**
  - **Radiobiology** - Biological effects at high doses are linear while at low doses are unpredictable likely attributed to variation in natural antioxidant capacity & repair mechanisms.
  - **Radiobiology** - Lower dose rates are less lethal implying repair mechanisms can keep up better till overwhelmed by rapid rate of damage production at higher dose rates
  - **Radiobiology** - Fractionation of exposure (broken up into a series vs. single time) decreases lethality implying that breaks between exposure allow repair processes to attempt to catch up.
  - **Water Chemistry** - H_2 shifts critical point where water decomposition, promoted by high LET component of radiation field, overcomes water reformation promoted by low LET component of radiation field (seen aqueous solutions of boric acid)

- **Management of Host Response**
  - **Radiobiology** - While hibernation doesn't decrease lethal dose, it does delays the radiation response. As well, CNS depressants extend survival time for lethal exposures from hours to days.
  - **Medical Gases** - Inducing a reduced metabolic state may prove to be an ideal therapy for various shock or trauma in which dramatic reduction in metabolic demands may be highly protective
  - **Medical Gases** - H_2S produces a "suspended animation-like" metabolic status & transiently and reversibly inhibits mitochondrial respiration
  - **DNA Repair** - DNA damage checkpoint arrests cell cycle progression to allow for repair prevention before replication of damage.

H_2 Protects Human Lymphocyte Cells from γ Irradiation

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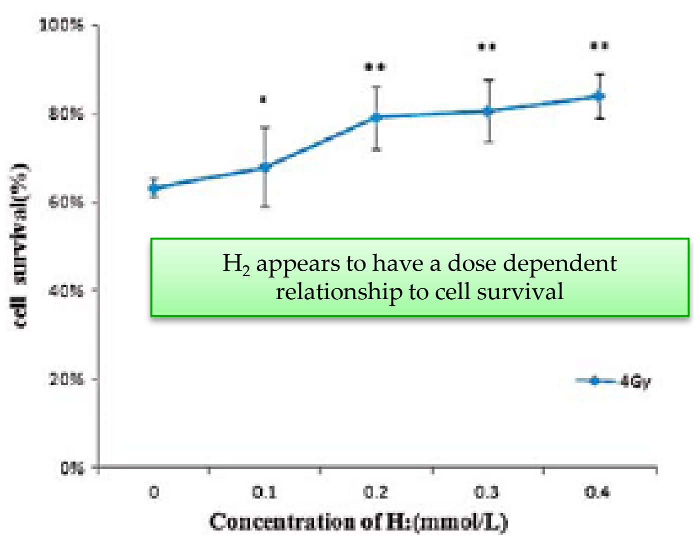
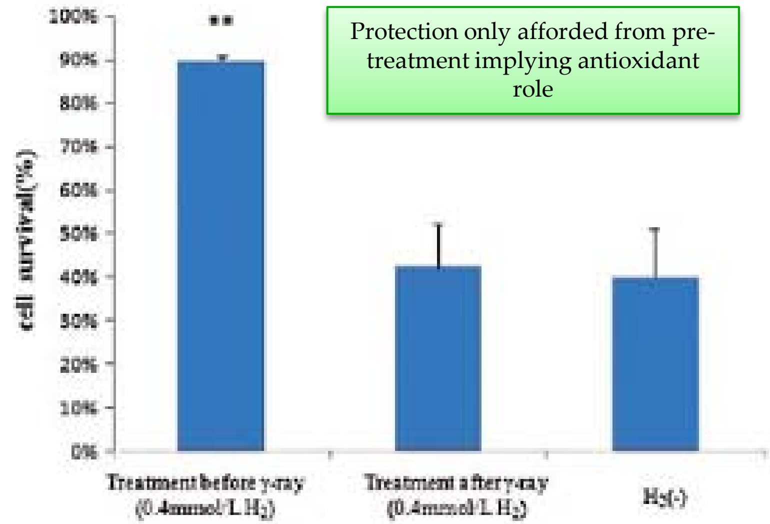
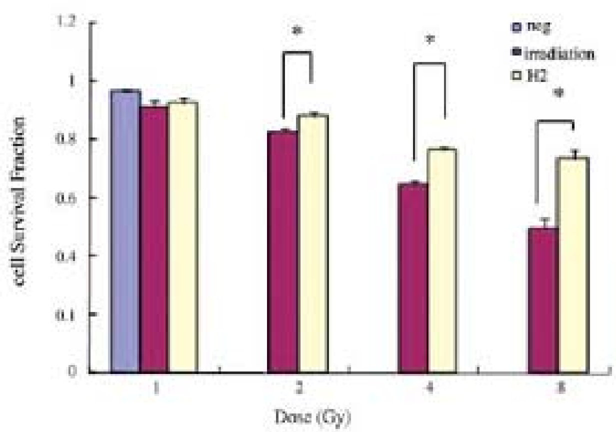


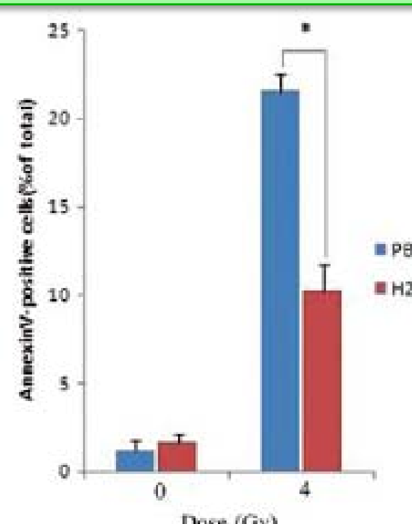
H_2 appears to have a dose dependent relationship to cell survival

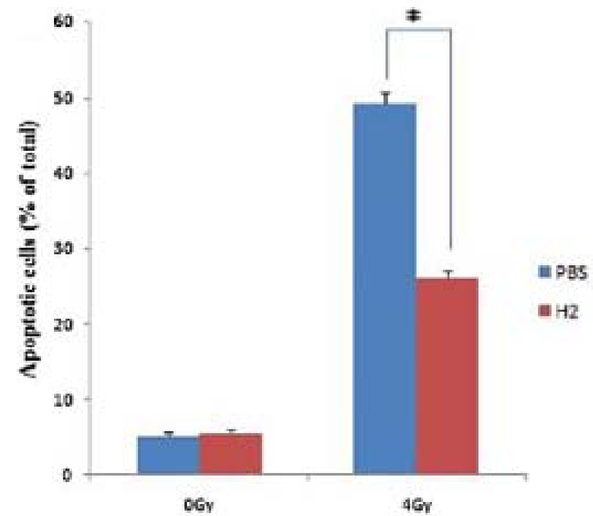


Protection only afforded from pre-treatment implying antioxidant role

Cell survival stems from a decrease of apoptosis either from enhanced repair or damage prevention







Qian L, Cao F, Cui J, Huang Y, Zhou X, Liu S, Cai J, "Radioprotective effect of Hydrogen in Cultured Cells and Mice", *Free Radical Research* 2010. **44(3)**:275-282.

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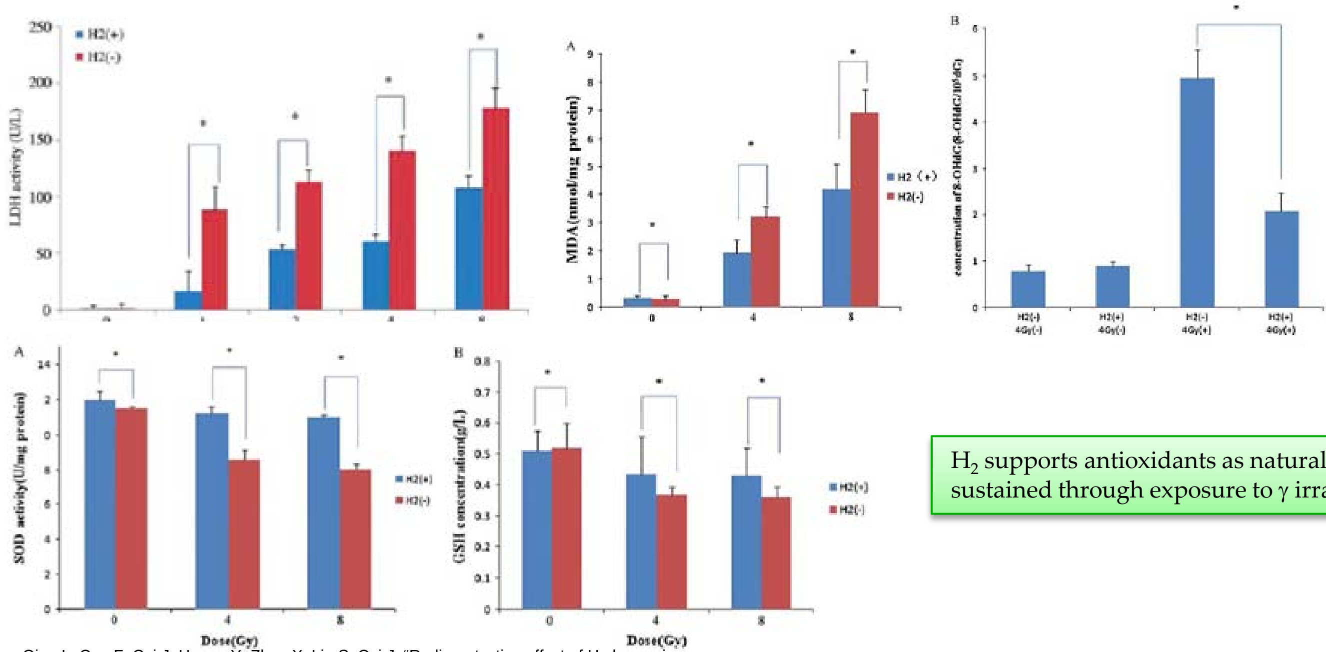
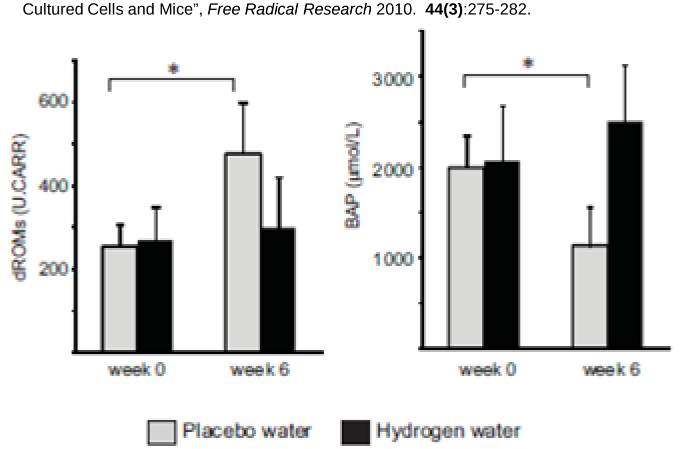
H_2 Protection by Damage Prevention



Qian L, Cao F, Cui J, Huang Y, Zhou X, Liu S, Cai J, "Radioprotective effect of Hydrogen in Cultured Cells and Mice", *Free Radical Research* 2010. **44(3)**:275-282.

H_2 reduced lipid & DNA oxidation in mice and cellular membrane oxidation in Human lymphocyte AHHH-1 cells exposed to γ irradiation

H_2 supports antioxidants as natural antioxidant levels (SOD & GSH) in mice are sustained through exposure to γ irradiation



H_2 decreases dROMs & increases BAP in patients undergoing radiotherapy

Nakao A, Toyoda Y, Sharma P, Evans M, Guthrie N, "Effectiveness of Hydrogen Rich Water on Antioxidant Status on Subjects with Potential Metabolic Syndrome—An Open Label Pilot Study", *J. Clin. Biochem. Nutr.* 2010. **46**:140-149.

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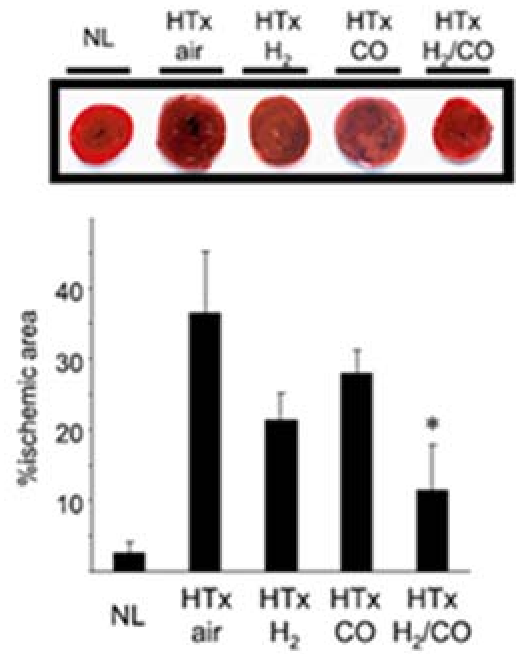
Suggested H_2 & CO Protective Mechanisms

H_2

Biochemical Mechanism	Notes
radical scavenging antioxidant	• selectively reduces hydroxyl radicals (•OH) and peroxynitrite ($ONOO^-$) but did not eliminate O_2^- or H_2O_2 when tested in *in vitro* [42]. • does not decrease the steady-state levels of nitric oxide (NO) [42] which may be beneficial as endogenous NO signaling pathways modulate pulmonary vascular tone and leukocyte/endothelial interactions [61]. • increases antioxidant enzymes such as catalase, superoxide dismutase or heme oxygenase-1 [45,46].
anti-apoptotic	• postulated to inhibit caspase-3 activation [62].
anti-inflammatory	• down-regulation of pro-inflammatory cytokines, such as interleukin (IL)-1 β, IL-6, chemokine (CC motif) ligand 2 and tumor necrosis factor-α (TNF-α) [63,64].

CO

Biochemical Mechanism	Notes
radical scavenging antioxidant	• binds to the heme moiety of mitochondrial cytochrom *c* oxidase. By binding to the heme, CO may prevent degradation of heme proteins which induce tissue injury by rapidly promoting peroxidation of the lipid membranes of cells [69, 70]. • reduces mitochondria-derived ROS thus resulting in lower levels of ROS generation in which an adaptive cellular response is triggered leading to cell survival rather than cell death [71-73]. • can induce HO-1 in cells to protect against injury [74-76]. Thus, detrimental excess of heme can be immediately removed by HO-1 enzymatic activity induced by CO.
decrease radiosensitivity	• impedes O_2 transport as it binds to hemoglobin with an affinity 240 times higher than that of O_2.



Nakao A, Kaczorowski DJ, Wang Y, Cardinal JS, Buchholz BM, Sugiomoto R, Tobita K, Lee S, Toyoda Y, Billiar TR, McCurry KR, "Amelioration of rat cardiac cold ischemia/reperfusion injury with inhaled hydrogen or carbon monoxide, or both", *J Heart Lung Transplant* 2010. **29**:544–553.

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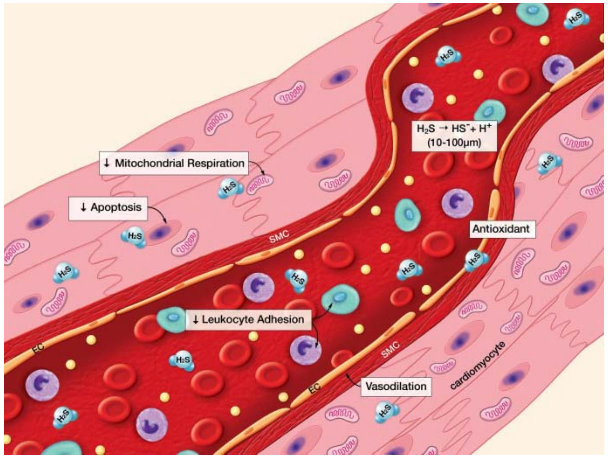
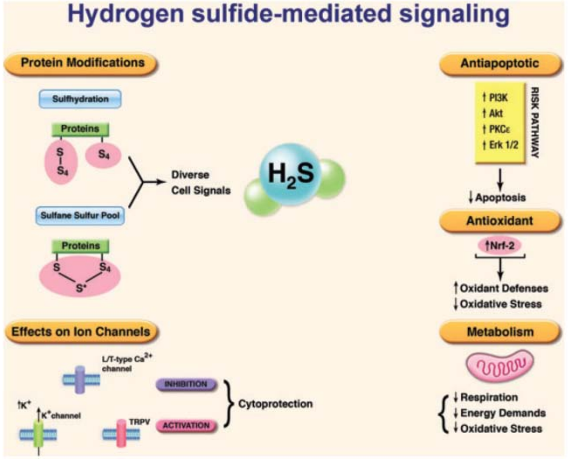
Suggested H_2S Protective Mechanisms

Biochemical Mechanism	Notes
radical scavenging antioxidant	• antioxidant inhibitor of peroxynitrite-mediated processes via activation of N-methly-D-aspartate (NMDA) receptors (Whiteman et al., 2004) • shield cultured neurons from oxidative damage by increasing levels of glutathione (Kimura et al., 2004) • induce upregulation of HO-1, anti-inflammatory and cytoprotective genes (Oh et al., 2006; Qingyou, 2004) • inhibits myeloperoxidase and destroys H_2O_2 (Laggner, et al., 2007)
anti-apoptotic	• reduces IR induced apoptosis via reduction of cleaved caspase-3 and cleaved poly (ADP-ribose) polymerase (PARP) (Sodha, 2008)
anti-inflammatory	• inhibit leukocyte adherence in the rat mesenteric microcirculation during vascular inflammation (Lefer, 2007)
decrease radiosensitivity	• vascorelaxtion and vasodilation of isolated blood vessels via vascular smooth muscle K_{ATP} channel-mediated hyperpolarization (Lefer, 2007; Nakao, et al., 2009) • transiently and reversibly inhibiting mitochondrial respiration (Lefer, 2007)
metabolic alteration	• produces a "suspended animation-like" metabolic status with hypothermia and reduced oxygen demand in pigs (who received it intravenously) (Simon, 2008) and mice (who received hydrogen sulfide via inhalation) (Blackstone, 2005; Blackstone, 2007).



Lefer DJ, "A new gaseous signaling molecule emerges: Cardioprotective role of hydrogen sulfide", In *Proceedings of the National Academy of Sciences*: November, 13, 2007; **104**(46):17907-17908.



Lefer DJ, King AL, "Cytoprotective actions of hydrogen sulfide in ischameia-reperfusion injury", *Exp Physiol* 2011.

Administration by Drinking, Injection, or Inhalation

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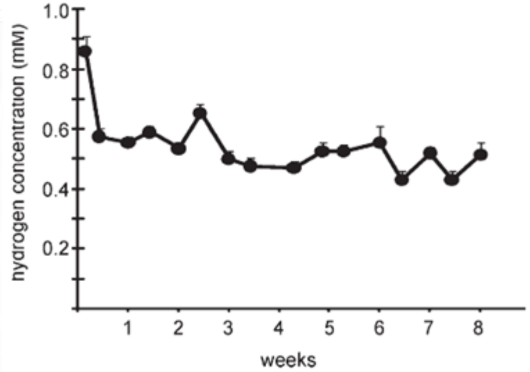
Generation By Chemical Reaction



Nakao, A., Toyoda, Y., Sharma, P., Evans, M. and Guthrie, N., "Effectiveness of Hydrogen Rich Water on Antioxidant Status on Subjects with Potential Metabolic Syndrome—An Open Label Pilot Study," *J. Clin. Biochem. Nutr.*, **46**, March 2010, pp. 140-149.

Magnesium Stick Insert

$Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2$

Dissolution In Solution

DRINKING WATER

Qian, L. et al., "Radioprotective effect of Hydrogen in Cultured Cells and Mice," *Free Radical Research*, **44(3)**, March 2010, pp. 275-282.

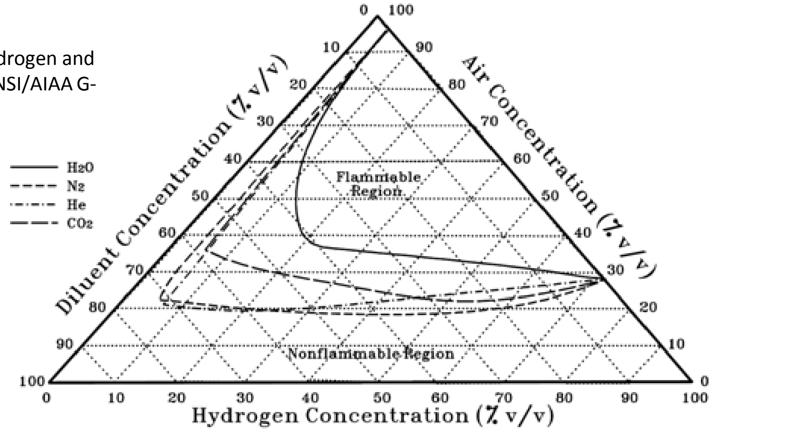
SALINE INJECTION

Non-Flammable Gas Mixtures As Additions to Atmosphere (Spacecraft/Station/Suit)

Hydreliox for Diving

- H_2: 49%
- He: 50%
- O_2: 1%

Guide to Safety of Hydrogen and Hydrogen Systems, ANSI/AIAA G-095-2004.



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Is Additional Protection Possible by Directly Enhancing DNA Damage Checkpoints & Repair Mechanisms?

1. damage sensor proteins don't preferentially bind to damage DNA.
   - *"DNA repair nor damage checkpoints should not be envisioned as operated by molecular switches. Rather, both processes are operative at all times, but the magnitudes of the repair or checkpoint reactions are amplified by the presence of DNA damage"*
   - *"Damage sensors not only bind to undamaged DNA in search of damage but they also contact undamaged DNA during specific binding…since the amount of undamaged DNA vastly exceeds that of damaged DNA, the DNA damage sensors spend far more time associated with undamaged DNA than with damaged DNA"*
2. Damage DNA appears to have a charged & thus can be acted on
   - *"The Comet Assay is based on the ability of negatively charged loops/fragments of DNA to be drawn through an agrose gel in response to an electric field. The extent of DNA migration depends directly upon the DNA damage present in the cells"*

Utilize electric or magnetic fields to enhance DNA damage detection or increase protein interaction rate?

- *nucleotide pool size has been indicated to have an important role in radiosensitivity → relate to ability of molecular diffusion within nucleotide pool?*
- *electric field centrifuge for assembly of damaged DNA damage?*
- *stirring of nucleotide pool to increase interaction frequency?*

Some Papers Yet to Review

- Mechanisms of DNA damage recognition and strand discrimination in human nucleotide excision repair (Nov. 2004)
- Checkpoint mechanisms at the intersection between DNA damage and repair (Sept. 2009)
- Surveillance mechanisms for monitoring chromosome breaks during mitosis and meiosis (April 2008)
- Mechanisms of DNA double-strand break repair by non-homologous end joining (June 2005)
- …….

Summary

1. High charge & energy (HZE) nature of space radiation makes it difficult to shield and particularly damaging to DNA. Posed concern of increasing effective dose as spacecraft travel faster.

2. Biological damage develops from a series of events that start with chemical modifications initiated by ionization (direct & indirect) and which lead to molecular transformations that manifest into biological diseases.

3. Hypothesized a biochemical approach to interrupt the damage process by interfering with chemical reactions and managing biological responses.

4. Hypothesized that medical gases can support natural repair & protection as: radical scavengers, tissue pre-conditioners, and signaling molecules in managing biological response to exposure. Posed idea if DNA repair mechanism & damage check points could be enhanced themselves?

5. Administration of a medical gas therapy in space applications appears feasible & reasonable.

6. Approach addresses biological damage from oxidative stress caused by reactive oxygen species (ROS) & therefore may have implications for other diseases related to oxidative stress such as:
   - cardiovascular disease
   - cancer
   - chronic inflammatory disease
   - hypertension
   - Ischemmia/reperfusion injury
   - acute respiratory distress syndrome
   - Parkinsons's & Alzheimer's
   - cataracts
   - aging

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Discussion & Questions

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Phone: 256-544-4557